Taste Thresholds of Butter Volatiles in Deodorized Butteroil Medium

SUMMARY—Taste thresholds of 31 volatile compounds found in butter were measured in deodorized butteroil and thresholds of seven volatiles were measured in fresh butter. Thresholds of mixtures of each of the major classes of volatile compounds (free fatty acids from C_2 through C_{12}, gamma-lactones from C_7 through C_{11}, even-numbered delta-lactones from C_8 through C_{14} and methyl ketones from C_3 through C_{13} except C_{12} were determined as well as thresholds of single compounds of these classes. Butyric acid, diacetyl, delta-decalactone, 2-nonanone, gamma-undecalactone and n-hexanal, oft-reported constituents of milk fat, had thresholds in butteroil of 0.66, 0.055, 1.4, 7.7, 0.95 and 0.19 ppm, respectively. The threshold of a mixture of free fatty acids from C_2 through C_{12} was 0.55 ppm. Synergistic interactions among methyl ketones and free fatty acids were pronounced and interactions among aldehydes were weak, while interactions among lactones were not apparent.

INTRODUCTION

MORE THAN 100 volatile compounds have been identified as natural constituents of butter or milk fat (Day et al., 1960; Forss et al., 1967; Jurriens et al., 1965; Langler et al., 1964, and Wong, 1963). A small number of these compounds are generally recognized as principal components of butter flavor. Different types of butter flavor concentrates are available from at least 41 commercial suppliers. None of these concentrates duplicates the complete natural flavor of butter, nor are all the compounds in these concentrates necessarily natural butter aroma constituents.

Human taste and odor thresholds of individual compounds are indexes of flavor (Patton et al. 1957). Thresholds of mixtures of compounds have been shown to complicate interpretations of flavor chemistry due to additive, synergistic and antagonistic interactions of mixtures (Day et al. 1963; Langler et al. 1964; Meijboom, 1964). Water thresholds of many butter volatiles are known. To complement the water threshold data, taste thresholds of key butter volatiles in butteroil medium and in butter itself were needed.

This study was undertaken to determine butteroil thresholds of individual compounds as well as mixtures of the prominent classes of volatile compounds found in butter. Compounds included were free fatty acids (even-numbered C_2 through $C_{18:1}$), delta-lactones (C_8, C_{10}, C_{12}, and C_{14}), gamma-lactones (C_7 through C_{11}), methyl ketones (C_3 through C_{11}, C_{13}, and C_{15}) and selected miscellaneous compounds which are known to be present in butter.

EXPERIMENTAL METHODS

Preparation of odor-free butteroil

Butteroil separated from fresh, melted sweet cream butter was filtered through Eaton & Dikeman No. 17 filter paper to remove the remaining free butter serum. The filtered oil was then vacuum steam-distilled in an all-glass apparatus designed to handle 10 to 12 L. of oil (Fig. 1). Earlier studies revealed that relatively high temperatures were required to remove butter flavor from the oil. In a typical vacuum steam distillation, melted oil was heated to 210° ± 10°C, held at this temperature for about 2 hr, then allowed to cool to 140°C. Antioxidant was added according to the method of Wyatt et al. (1965). The pressure above the oil, which increased with temperature, ranged from 9 to 23 mm (Hg), and was 1 mm or less at the pump end of the system. The amount of water distilled was 1/4 to 1/2 the oil volume.

Volatile compounds, as well as cholesterol and carotenoids, were removed by the distillation. The deodorized oil had a faint odor and a slightly sweet or nutty taste. A recognizable butter flavor, however, was not detectable.

Purification of compounds tested

For flavor studies, reagent grade chemicals whose normal boiling points were below 230°C were vacuum-distilled in an all-glass 10 to 50-ml capacity distillation apparatus. Lauric, myristic, palmitic and oleic acids were purified by several recrystallizations from ethanol-water. Other high boiling compounds were purified by preparative GLC, using a 1/4 inch × 4.5 ft aluminum column containing 80/120 mesh glass beads coated with 0.1% Apiezon H. Purity of the liquids, as determined by gas chromatography (150 feet × 0.01 in. I.D. stainless steel capillary coated with Ucon), was better than 99.8% except for 2-pentanone (99.6%).

Testing procedures

Glassware used in all tests was thoroughly cleaned and carefully inspected to exclude containers which had residual stains or odors. Stock solutions were prepared in 100-ml volumetric flasks by dissolving calculated amounts of purified compounds in deodorized oil at 38° ± 4°C and bringing to volume; four or five dilutions to lower concentrations were made from each stock solution. Compounds whose thresholds were measured in water were brought into solution by first dissolving the compound in ethanol and then dispersing the ethanol solution into water (subthreshold ethanol concentration was maintained).

The flavor tests were conducted by the procedure described by Wyatt et al. (1965).

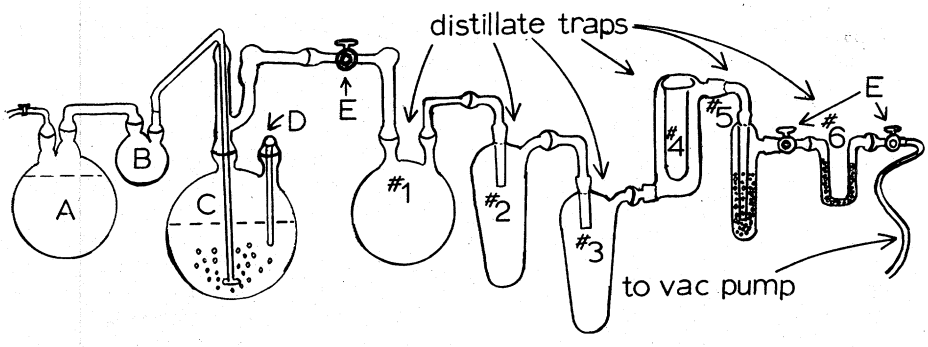

Fig. 1—Vacuum steam distillation apparatus used. A—steam reservoir, B—safety trap, C—substrate receptacle, D—thermometer well, E—stockcocks; traps #1 and #2—dry ice-acetone cooled; traps #3 through #6—liquid nitrogen cooled traps (traps #5 and #6 contain glass beads).

Table 1—Taste thresholds of selected butter volatiles measured in deodorized butteroil

Compound	Concentration in ppm: Threshold	Concentration in ppm: Approximate level in fresh butter[1]
Butyric acid	0.66	9 to 38
Caproic acid	2.5[2]	6 to 18
Diacetyl	0.055	0.02 to 0.1
Dimethyl sulfide	0.009	0.02
Delta-decalactone	1.4	0.01 to 9
Ethyl acetate	22	4
Ethyl butyrate	0.60	<0.2
Ethyl hexanoate	0.85	<0.1
Acetaldehyde	0.11	0.7
n-Hexanal	0.19	0.14
n-Heptanal	0.75	0.16
n-Nonanal	1.0[3]	0.07

[1] Concentration values were from the following sources: fatty acids, Iyer et al. (1967); aldehydes, Day et al. (1960); and delta-decalactone by Forss et al. (1967) (0.01) and Jurriens et al. (1965). Other concentrations were estimated from GLC analysis of butter volatiles (unpublished data, T. J. Siek).
[2] From Patton (1964).
[3] From Hvolby (1962).

Table 2—Taste thresholds of free fatty acids in deodorized butteroil—individual compounds and mixtures

Fatty acid	Concentrations in ppm: Individual threshold[1]	Fatty acid in mixture at the mixture threshold[2]: I	Fatty acid in mixture at the mixture threshold[2]: II
C_2	7.0	0.002	0.003
C_4	0.66	0.07	0.10
C_6	2.5	0.03	0.06
C_8	350	0.05	0.08
C_{10}	200	0.11	0.17
C_{12}	700	0.29	0.45
C_2–C_{12}	1260	0.55 = mixture threshold	
C_{14}	5000		814
C_{16}	10000		2203
C_{18}	15000		617
$C_{18:1}$	8000		1315
C_2–$C_{18:1}$	39260		5000 = mixture threshold

[1] Individual thresholds were taken from Feron et al. (1961) for C_6 through $C_{18:1}$; their value for C_4 was 0.60.
[2] Column I was a mixture through C_{12}; column II contained all fatty acids listed.

Four or five dilutions of the compound being tested were served to tasters along with a labeled blank (zero concentration) and a coded blank. The panel of judges consisted of 28 members of the Food Science and Technology staff with 20 members used per test; oil samples were served at 42° ± 3°C; and judges were not pre-selected for taste acuity, but were experienced in serving on flavor panels.

In the flavor booths, judges were asked to taste the two reference samples (zero concentration and the highest concentration) and then to taste at random the coded samples containing the flavor compound. The judges marked their ballot "plus" when the compound in question was detected in a sample. No time limit was imposed during tasting. If panel members misjudged the coded blank and/or coded maximum concentration sample, their ballot was excluded from the results. Otherwise, each "plus" answer was recorded. Taste tests were conducted within a week after the sample purification. Solutions of compounds tested were made on the day of the test. The 50% positive response level used by Patton et al. (1957) was calculated, so that direct comparisons might be made with reported 50% thresholds. Threshold concentrations are defined as concentrations of compounds in a given medium in parts per million.

For comparison, several thresholds were measured in fresh sweet cream butter. The butter samples were served from the refrigerator (4°C) as butter patties. Mixture solutions of several classes of butter volatiles were also prepared for testing. Mixture thresholds were established in the same manner as single-compound thresholds, the mixture threshold being the total volatile concentration which could be detected. Ratios of individual compounds were constant in each dilution of the mixture.

Threshold determinations usually required one or more preliminary tests to find the appropriate concentration range. In practice, panelists often sniffed all samples and tasted only those they could not categorize by sniffing. Panelists could usually detect one or two concentrations lower by tasting than they could by sniffing only. Meijboom (1964) found that for 31 aldehydes, threshold values for taste (measured in paraffin oil) were in all cases lower than those for odor.

Table 3—Taste thresholds of methyl ketones in deodorized butteroil—individual compounds and mixtures

Carbon number	Concentration in ppm: Individual threshold[1]	Concentration in ppm: Ketone in mixture at mixture threshold
C_3	125	0.09
C_4	30	0.09
C_5	61	2.6
C_6	—	0.09
C_7	15	3.5
C_8	2.5	0.09
C_9	7.7	2.6
C_{10}	11	0.09
C_{11}	100	5.3
C_{13}	182	3.5
C_{15}	—	5.3
C_3 to C_{15}		23 = mixture threshold

[1] Values obtained by Hvolby (1962) for C_4, C_8, C_{10}, C_{11}, and C_{13} were 30, 5, 10, 10, and 500 respectively.

RESULTS & DISCUSSION

Individual thresholds

Taste thresholds of several butter compounds are presented in Table 1 along with their reported or estimated concentrations in butter. Other individual thresholds are shown in Tables 2, 3, 4, and 5.

The threshold of delta-decalactone was measured five times (on five days) with some variation of panel members in each test. The threshold obtained was 1.4 ± 1.1 (σ). Greater precision was noted in repeated tests with other compounds: for 2-pentanone the threshold was 61 ± 3; gamma-undecalactone, 0.95 ± 0.22, and *n*-hexanal, 0.016 ± 0.003 (in water). Thresholds depend greatly on expertise and size of the panel used (Amerine et al. 1965, p. 182); thus differences in taste thresholds from different laboratories are probably due to differences in make-up of the panel. By observing general trends of a homologous series (Table 3) more validity can be ascribed to individual threshold values, and from these values a measure of relative flavor potential can be ascertained.

Table 4—Taste thresholds of normal aldehydes in deodorized butteroil—individual compounds and mixture

Carbon number	Concentration in ppm: Individual threshold	Concentration in ppm: Aldehyde in mixture at mixture threshold
C_5	0.30	0.16
C_6	0.19	0.16
C_7	0.75	0.16
C_8	0.9[1]	0.16
		0.64 = mixture threshold

[1] From Lea and Swoboda (1958); their value for *n*-hexanal was 0.3.

Table 5—Taste thresholds of lactones in deodorized butteroil—individual compounds and mixtures

Lactone	Concentration in ppm: Individual threshold	Concentration in ppm: Lactone in mixture at mixture threshold[1]
γ-C_6	8.0	—
γ-C_7	3.4	0.47
γ-C_8	3.5	0.94
γ-C_9	2.4	0.94
γ-C_{10}	1.0	0.94
γ-C_{11}	0.95	0.94
		4.3 = mixture threshold
δ-C_8	3.0	0.54
δ-C_{10}	1.4	2.7
δ-C_{12}	95	5.4
δ-C_{14}	500	5.4
		14 = mixture threshold

[1] Gamma- and delta-lactone mixtures were measured separately.

Thresholds of mixtures

Thresholds of individual compounds and thresholds of mixtures of several classes of butter volatiles are given in Tables 2, 3, 4 and 5 (note that in Tables 2 to 5, individual compound concentrations are given above the mixture threshold concentration and individual thresholds are in a separate column). Methyl ketone, free fatty acid and aldehyde homologous series mixtures exhibit results similar to those reported by Langler et al. (1964). Interaction in the mixtures is apparent especially with fatty acid and methyl ketone mixtures (Tables 2 and 3), as at the mixture threshold, concentrations of individual compounds are subthreshold. Langler et al. (1964) refer to such effects as "synergistic." The synergistic effect was not pronounced with aldehydes and was not evident among lactones.

Table 6 gives the water, oil and butter taste thresholds of several selected compounds obtained in this study and by other investigators. The data show that oil thresholds are generally higher than water thresholds. Thresholds in butter are closer to oil thresholds than water thresholds. Factors influencing water and oil threshold differences have been discussed previ-

CONCLUSIONS

Among the thresholds measured, those of several butter volatiles are lower (or nearly so) than their reported concentration in butter, and thus would be expected to contribute to sweet cream butter flavor. Compounds that fall into this group are diacetyl, butyric and caproic acids, hexanal, acetaldehyde, dimethyl sulfide and possibly delta-decalactone. Levels of 2-hepatanone and 2-nonanone (Langler et al. 1964) could be high enough to influence flavor due to synergistic interactions. The aroma and flavor of fresh cream butter depend on a concentration balance of low threshold compounds reported herein (plus possibly some volatiles not as yet identified in butter), with little contribution to flavor by high threshold compounds. Synergistic interactions exhibited by mixtures probably play an important role in giving butter its unique flavor and aroma.

Table 6—Taste thresholds of representative volatile compounds in different media

Compound	Threshold concentration in ppm			
	Water	Oil	Milk	Butter
Ethyl acetate	6.6	22	4.7	—
Ethyl butyrate	0.015	0.60	0.016	—
Dimethyl sulfide	—	0.009	0.019[1]	0.17
Diacetyl	0.0054	0.055	0.014	0.032
2-Octanone	0.15	2.5	—	3.4
2-Decanone	0.19	11	—	9.3
Delta-decalactone	0.14	1.4		
Acetic acid	22	7.0		
Butyric acid	6.2	0.66	25[2]	
Caproic acid	15	2.5[2]	14	
Caprylic acid	5.8[2]	350[2]	23[3]	
Capric acid	3.5[2]	200[2]	28[2]	
n-Heptanol[4]	2.4	20	—	10
n-Dodecanal[4]	0.011	0.75[5]		
Acetaldehyde	1.3[6]	0.11		
n-Pentanal	0.07	0.30	0.13[7]	
n-Hexanal	0.016	0.19	0.05[7]	0.80
n-Heptanal	0.031	0.75	0.12[7]	0.90

[1] From Reddy et al. (1967).
[2] From Patton (1964); his values for acetic, butyric and caproic acids were 54, 6.8 and 5.4 respectively.
[3] Concentration that gives a rancid flavor (Scanlan et al. 1965); butyric and caproic acid values (rancid flavor) were 46 and 30 respectively.
[4] Not indigenous to butter.
[5] Lea et al. (1958) reported 0.9.
[6] From Berg et al. (1955).
[7] From Day et al. (1963).

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